Synthesis and aggregation behaviour of two-headed surfactants containing the urocanic acid moiety

Sophie Franceschi,[a] Valérie Andreu,[a] Nancy de Viguerie,[a] Monique Riviere,*[,a] Armand Lattes[a] and André Moisand[b]

[a] *Laboratoire des IMRCP, Université Paul Sabatier, 118, route de Narbonne, 31062 Toulouse cedex, France*
[b] *Institut de Pharmacologie et Biologie Structurale, CNRS, 205, route de Narbonne, 31077 Toulouse cedex, France*

Urocanic acid (3-[1*H*-imidazol-4-yl]propenoic acid) has attracted great interest in photobiology for many years. We describe here the synthesis of three bolaamphiphiles with two urocanic acid heads having the general structure: UA—X—UA, where UA denotes urocanic acid and X is an alkyl chain of varying length. We show that micellisation occurs with the bolaamphiphile whose alkyl chain length is sixteen carbon atoms. For compounds having a shorter chain length, light scattering and electron microscopy suggest the formation of vesicles. Compared to urocanic acid, the bolaamphiphiles, which can form aggregates in aqueous solution, may act differently on membranes and be used in formulations more easily.

Synthèse et agrégation d'amphiphiles à deux têtes comportant un motif acide urocanique. L'acide 3-[1*H*-imidazol-4-yl]propénoïque, ou acide urocanique, possède des propriétés photobiologiques intéressantes. Des bolaamphiphiles dérivés de cet acide sont susceptibles de présenter des avantages sur le plan de la formulation ainsi qu'un comportement différent au niveau des membranes. Trois bolaamphiphiles de structure AU—X—AU, où AU représente le motif acide urocanique et X est un segment lipophile de longueur variable, ont été synthétisés et leurs phénomènes d'agrégation en solution aqueuse étudiés. Seul le composé présentant une longueur de chaîne de seize atomes de carbone forme des micelles. Pour les bolaamphiphiles ayant des chaînes hydrocarbonées plus courtes la formation de vésicules est observée par diffusion de la lumière et microscopie électronique.

Urocanic acid (3-[1*H*-imidazol-4-yl]propenoic acid) has been for a long time of great interest in photobiology.[1,2] The two isomers *E* and *Z* (Fig. 1) are found in the epidermis. The *E* isomer, formed by deamination of histidine and not catabolised (absence of urocanase in the skin), accumulates in the Stratum corneum[3] and is partly excreted in the sweat.[4] Under UV irradiation, (*E*)-urocanic acid is isomerised in the skin to produce a mixture of the two isomers[5] in almost equal quantities. These two isomers, possessing a large absorption band with a maximum at 270 nm and a strong molar extinction coefficient, have remarkable biological properties. Indeed, as a major chromophore present in the skin, urocanic acid acts as a photoprotective agent and for the same reason has potential applications in cosmetology. However, urocanic acid can also lead to harmful effects because of [2 + 2] cycloadditions,[6] photooxidations[7] and other photochemical interactions[8] with various compounds of biological importance. More importantly, due to its immunosuppressive activity[9–11] (attributed to the *Z* isomer) it is susceptible to being involved in the process of skin photocancerisation.

The relationship between the formation of (*Z*)-urocanic acid and the effects observed on the immunity system are complex and many studies are under way to elucidate the mechanism of action of urocanic acid.[12,13] Despite the fact that this naturally produced compound has immunosuppressive effects, it remains very interesting because it can have clinical applications against the phenomenon of transplant rejection or for the treatment of skin diseases like psoriasis. Therefore, we have been interested in various derivatives of this compound, particularly long chain analogues. While keeping a photoprotective activity they may, due to their hydrophobic character, operate differently on the membranes. Also, from the galenic point of view they can be introduced more easily into formulations, whereas urocanic acid is practically insoluble in organic and aqueous media and difficult to formulate.

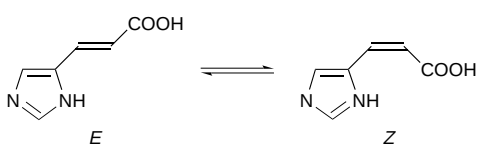


Fig. 1 *E* and *Z* isomers of urocanic acid

In a preceding work we observed that long chain esters of urocanic acid present good photoprotective qualities:[14] (*i*) their spectral properties are analogous to those of urocanic acid; (*ii*) they are anchored in aggregates in organized media (micellar solutions and microemulsions), which by extrapolation suggests similar anchoring in biological media; and (*iii*) their photostability in organized media is good, with urocanic acid being the only degradation product after five days of irradiation. Because of these useful properties, we chose to explore other kinds of long chain derivatives, the bolaamphiphiles. A bolaamphiphile[15] or bolyte is constituted of two polar heads and a hydrophobic spacer. Bolaamphiphiles are capable of organizing in an aqueous environment to form aggregates of various morphologies:[16] spheres (Fig. 2a), small spherocylinders, large cylinders, discs, lamellae and vesicles (Fig. 2b). The shape of the aggregates depends on the length of the spacer and its rigidity or flexibility (influenced by the presence of unsaturation or ring structures). The formation of vesicles

* Fax: (+33) 5.61.25.17.33; E-mail: mriviere@iris.ups-tlse.fr

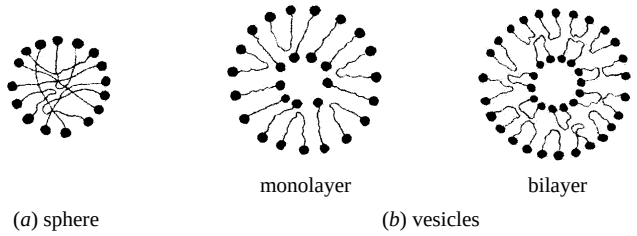


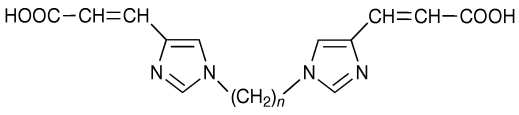
Fig. 2 Aggregation patterns of bolaamphiphiles in (*a*) spheres and (*b*) monolayer and bilayer vesicles

from such molecules is of interest since their cellular toxicity might be very low, not being able to penetrate through membranes, and therefore they could be used clinically. Such vesicles can be considered as models of stable and functionalized membranes.

The synthesis of bolaform molecules and the study of their organization have been described in the literature. In particular, the aggregation behaviour in aqueous solutions of bolaamphiphiles, composed of two ammonium head groups and a single hydrocarbon chain, have been studied.[17–20] From surface tension measurements and spectral property changes, the authors conclude that compounds having a spacer of less than 12 carbon atoms behave as simple electrolytes while compounds having spacers of 16 to 22 carbon atoms form micelles. Okahata and Kunitake[21] have synthesized a great variety of amphiphiles constituted of two heads and a long chain. They observed by electronic microscopy the formation of globules, ribbons, monolayer discs and vesicles. Fuhrhop and Fritsch[22] have investigated the extraction of natural bolaamphiphiles so as to study their cellular function. Among them, the bolaamphiphiles constituting the membrane of a microorganism have been isolated.[23] The very rigid membranes allow this microorganism to survive under drastic conditions of pH, temperature and pressure. These authors have also undertaken the synthesis of more than one hundred bolaamphiphiles, for the purpose of forming functionalized vesicles.[24,25] Garelli-Calvet *et al.*[26] have synthesized bolaamphiphiles having sugar groups as polar heads (bis-gluconamides and bis-lactobionamides). These compounds organize into micelles or vesicles,[27] depending upon the length of the hydrocarbon chain. The aggregates formed are able to solubilize hydrophobic compounds like fatty acids and have proven to be not denaturing for lipoxygenases. These authors have also synthesized derivatives of disulfonic naphthalene acids[28] as bolaamphiphiles that organise into vesicles. These compounds present an anti-HIV-1 activity more interesting than that of the corresponding monopolar derivatives, as well as a lower toxicity attributed to the lack of a rupturing effect on cellular membranes.

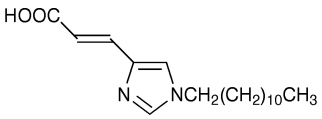
Other ionic and nonionic bolytes have also been described in the literature. The head group of nonionic bolaamphiphiles includes: aroyl azide diamide,[29] morpholine,[30] arborols,[31,32] polyoxyethylene,[33,34] and in case of an unsymmetrical bolaamphiphile one carboxylic acid head and the other a maleic or succinic anhydride.[35] The ionic head groups studied include sodium sulfate,[36] pyridinium,[37,38] phosphate,[39] carboxylate[40], an L-lysine and an amino group[41] in the case of an unsymmetrical molecule.

In this paper, we first describe the synthesis of three symmetrical bolaamphiphiles having urocanic acid head groups and a hydrocarbon spacer of 8, 12 or 16 carbon atoms:



$n = 8$ UAC_8UA
$n = 12$ $UAC_{12}UA$
$n = 16$ $UAC_{16}UA$

We then discuss the behaviour of these bolaamphiphiles at the water–air interface as studied by surface tension measurements, and their properties in an aqueous environment as explored by conductometry, spectrophotometry, light scattering and electron microscopy. The results are compared with those obtained for the monopolar derivative, $C_{12}UA$:



$C_{12}UA$

Depending upon the pH of the aqueous medium, the above four compounds can be ionic or nonionic. Therefore the aggregate formed will be influenced by both the pH and the length of the alkyl chain.

Synthesis

We have used for this synthesis the method developed earlier in our laboratory for the N_τ alkylation of methyl urocanate.[42] Under solid–liquid phase-transfer conditions, the dibromoalkane is allowed to react with (*E*)-urocanic acid in the presence of potassium carbonate, with a crown ether acting as the catalyst. After purification the methyl esters of the three bolytes are obtained with 52, 45 and 65% yields, respectively. The ester function is then hydrolysed under basic conditions (Fig. 3). The purity of all the compounds has been carefully checked. Only $UAC_{16}UA$ contained significant impurities (3–4%), in the form of the monomethyl ester arising from an incomplete hydrolysis.

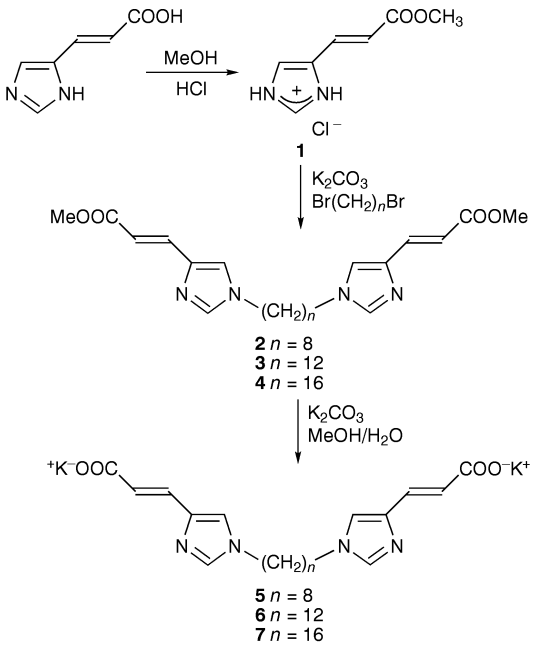


Fig. 3 Synthesis of urocanic bolaamphiphiles

Study of Self-organization

Micelles

For each bolaamphiphile we tried to identify which type of aggregates form spontaneously in an aqueous solution. First, the solubility of these four compounds was determined in water at various pH (Table 1). In a neutral solution the solubility is low. Under basic conditions, $C_{12}UA$ behaves as a single-chain ionic surfactant with a Krafft temperature of 25 °C. $UAC_{16}UA$ behaves similarly and also has a Krafft temperature of 25 °C. For the two other bolytes the solubility is high. Based on these results, self-organization studies have been undertaken at pH 13.2 and 30 °C using three methods: surface tension, conductivity and UV absorbance measurements. Concentrations from 10^{-5} to 10^{-2} mol l^{-1} have been chosen for this study. As a reference the compound $C_{12}UA$ (one-headed amphiphile) has been studied.

The results showing the evolution of the surface tension, the conductivity and the optical density with the concentration of amphiphile in aqueous solution at pH 13.2 are given in Figs. 4, 5 and 6.

The shape of the surface tension curve (Fig. 4) of solutions of UAC_8UA between 10^{-5} and 10^{-2} mol l^{-1} shows that from 5×10^{-5} to 5×10^{-4} mol l^{-1}, the surface tension does not decrease, possibly due to the large solubility of this amphiphile in water; indeed, compared to the two other bolaamphiphiles, it is less hydrophobic. Above 10^{-3} mol l^{-1}, the surface tension decreases strongly until it reaches a value of 35 mN m^{-1} at 10^{-2} mol l^{-1}, implying that UAC_8UA is positioned at the air–water interface. Above this concentration UAC_8UA may form micelles.

The curve obtained for the bolaamphiphile $UAC_{12}UA$ shows a break point at a concentration of 4×10^{-4} mol l^{-1}, which was taken as indicating a change of organization at the air–water interface. However, a real plateau is not observed and the surface tension continues to decrease for higher concentrations, so this behaviour is not characteristic of micelle formation.

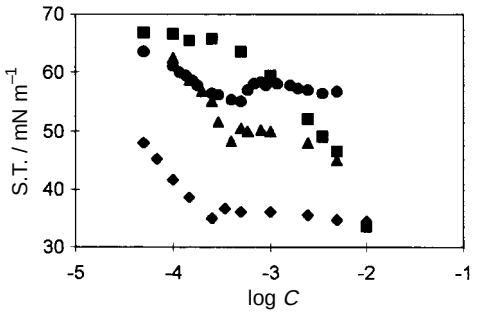


Fig. 4 Surface tension *versus* concentration of urocanic acid amphiphiles (◆ UAC_{12}, ■ UAC_8UA, ▲ $UAC_{12}UA$, ● $UAC_{16}UA$) in aqueous solutions (pH 13.2) at 30 °C

As indicated above, $UAC_{16}UA$ contains a small amount of monomethyl ester. It seems, therefore, to form micelles at a concentration around 4.7×10^{-4} mol l^{-1} (c.m.c.). We notice that the amplitude of variation of the surface tension is rather small (from 72 to 55 mN m^{-1}), compared to the behaviour shown by classical surfactants. The surface tension curve presents a minimum that may be due to the monomethyl ester impurity. Nevertheless, the surface tension curves of UAC_{12} and $UAC_{12}UA$ also show a slight minimum; to our knowledge these compounds do not contain the monomethyl ester.

Conductometry (Fig. 5) and spectrophotometry (Fig. 6) studies confirm the results obtained by tensiometry: namely, no micellization phenomenon for UAC_8UA, a change of organisation for $UAC_{12}UA$ and a critical micellar concentration (5×10^{-4} mol l^{-1}) for $UAC_{16}UA$.

The concentrations at which a change of organisation is observed (determined by the above three methods) and the measured surface tension minima are reported in Table 2.

Other aggregates: vesicles

We also investigated whether these compounds are able to form vesicles in aqueous solution, using two methods that provide information on the shape as well as the size of the aggregates. The first method uses light scattering. The measure of the light scattered by particles illuminated with a

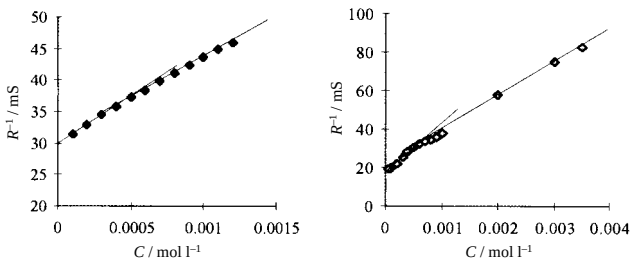


Fig. 5 Conductivity *versus* concentration of urocanic acid amphiphile aqueous solutions (pH 13.2) at 30 °C (◆ $UAC_{12}UA$, ◇ $UAC_{16}UA$)

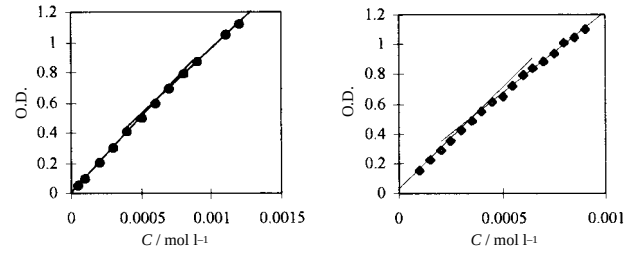


Fig. 6 Optical density *versus* concentration of urocanic acid amphiphile aqueous solutions (pH 13.2) at 30 °C. λ = 322 nm for $UAC_{12}UA$ (●) and λ = 320 nm for $UAC_{16}UA$ (◆)

Table 1 Values of the solubility (in water and in aqueous NaOH solution), the Krafft temperature (T_{krafft}), the molar extinction coefficient (ε), and the maximum wavelength (λ_{max})

Compound	Solubility in water at 30 °C/ mol l^{-1}	Solubility in aqueous NaOH (pH 13.2) at 30 °C/moll^{-1}	T_{krafft}/°C	ε in aqueous NaOH (pH 13.2)/ 1 mol^{-1} cm^{-1}	λ_{max} in aqueous NaOH (pH 13.2)/ nm
$C_{12}UA$	9.3×10^{-5}	$>5 \times 10^{-1}$	25	13 391	280
UAC_8UA	1.5×10^{-4}	7.9×10^{-2}	<5	42 328	280
$UAC_{12}UA$	3.6×10^{-5}	4.1×10^{-2}	<5	40 533	280
$UAC_{16}UA$	5.5×10^{-6}	9.6×10^{-3}	25	38 530	280

Table 2 Values of the concentrations (mol l^{-1}) (as determined by various methods) at which a change of organization is observed: γ_{min} (mN m^{-1}) is the measured surface tension at these concentrations

Compound	Tensiometry	Conductometry	Spectrophotometry	γ_{min}
$C_{12}UA$	1.8×10^{-4}	ND	ND	37
UAC_8UA	no	no	no	no
$UAC_{12}UA$	4×10^{-4}	5.4×10^{-4}	5.7×10^{-4}	48
$UAC_{16}UA$	4.7×10^{-4}	5×10^{-4}	4.7×10^{-4}	55

ND: not determined. no: no change observed.

laser beam allows one to determine the size and the distribution of objects in solution. Particles having diameters between 3 and 3000 nm can be detected by this technique. The second method is electron microscopy. It allows one to visualise directly aggregates by colour-staining techniques that provide better contrast. This method allows the observation of aggregates like vesicles, but not micelles because they are dynamic and are of small size. This experimental technique can, however, deform the aggregates.

Typical diameters of different aggregates formed by various one-headed amphiphiles are given in Table 3 and come from the literature.[43]

For vesicle preparation,[44–48] we first used a method described for the preparation of giant polydisperse vesicles of phospholipids, namely the drying-rehydratation method.[49] In this procedure, phospholipids are dissolved in an organic solvent. The solvent is then removed in order to maximize the film area. Water is then slowly added at 70 °C and the flask is shaken for few seconds. However, no aggregates were obtained with the urocanic bolaamphiphiles when using this procedure. We could suppose that this method, never reported in the literature for synthetic bolaamphiphiles, was not suitable for compounds so different in structure from phospholipids.

Thus we have used the only literature method for bolaamphiphiles. In this procedure, solutions of the bolaamphiphiles at pH 13.2 were sonicated. By light scattering, we observed aggregates of approximately 200 nm diameter for UAC_8UA and $UAC_{12}UA$ and also very small objects ($\approx$2 nm) difficult to identify. Possibly, these observations can be explained by a competition between micelle and vesicle formation from an homogeneous solution.

The effect of pH on aggregate formation has been studied, using a pH 10 solution of sodium hydroxyde and pure water. After sonication, the solutions were analysed by light scattering. The diameters of all the objects observed were near 200 nm.

The effect of variations of the power output, the % duty cycle (number of pulses during a fixed time) and the duration of the ultrasonic application have also been studied in the case of a solution of $UAC_{12}UA$ in pure water. Since no differences were observed, the following sonication parameters have been used: 15 min duration of the ultrasonic application; 110 watt power output; 80% duty cycle.

Table 4 shows the diameters and the size distribution of the aggregates (% aggregates of a given size) of the bolaamphiphiles UAC_8UA and $UAC_{12}UA$ in pure water and in pH 10 and pH 14 sodium hydroxide solutions, observed by light scattering. The results are the average of 10 to 20 experiments; for each size determination the standard deviation is about 20%.

For these two compounds, on going from pH 14 to pH 10 the small-size particles disappeared and the formation of 200 nm vesicles is favoured. An increase in the size of vesicles on going from higher to lower pH has been observed also for double-chain surfactants with two carboxylate head groups by Engberts and co-workers[50] and by Jaeger and Brown.[51] Knowing that the pK of dodecanoic acid in the bilayer form is about 8[52] and assuming that in vesicles the second pK of dicarboxylic surfactants is superior to 8.5, the latter authors attributed the pH effect on the size of vesicles to the presence of a greater proportion of carboxyl groups among the carboxyl and carboxylate head groups. An incomplete deprotonation can produce hydrogen bonding between the polar heads. Thus pH seems to be an important factor in vesicle formation.[53] Our results are consistent with this explanation.

In the case of $UAC_{16}UA$, light scattering has not allowed us to conclude that vesicles are present.

The formation of vesicles for all three bolaamphiphiles was also investigated by electron microscopy. The micrographs show more or less spherical particles with well-defined outlines. So this technique confirms that the three urocanic bolytes are able to form vesicles. pH Variations do not affect the shape and the size of the observed vesicles. The small particles of 1 or 2 nm size seen by light scattering are not observed by electron microscopy because of their small dimensions. Monolayer vesicles (about 100 nm diameter) are observed with the three urocanic bolaamphiphiles, as well as larger species probably formed by coalescence of vesicles.

For $UAC_{16}UA$, electron micrographs show vesicles in much smaller amounts compared to the two other com-

Table 3 Diameters of the various aggregates formed by monopolar amphiphiles.

Aggregate	Micelles	Small unilamellar vesicles	Large unilamellar vesicles	Giant vesicles
Diameter/nm	5	30–50	100–200	5000–200 000

Table 4 Diameters (ϕ) and size distribution (%) of aggregates of the bolaamphiphiles UAC_8UA and $UAC_{12}UA$ in pure water and in pH 10 and pH 14 sodium hydroxide solutions (observed by light scattering)

UAC_8UA						$UAC_{12}UA$					
Pure water		NaOH soln pH 10		NaOH soln pH 14		Pure water		NaOH soln pH 10		NaOH soln pH 14	
ϕ/nm	%	ϕ/nm	%	ϕ/nm	%	ϕ/nm	%	ϕ/nm	%	ϕ/nm	%
				1	49					2	54
12	1	23	2	64	14						
212	99	189	98	201	37	182	100	186	100	204	46

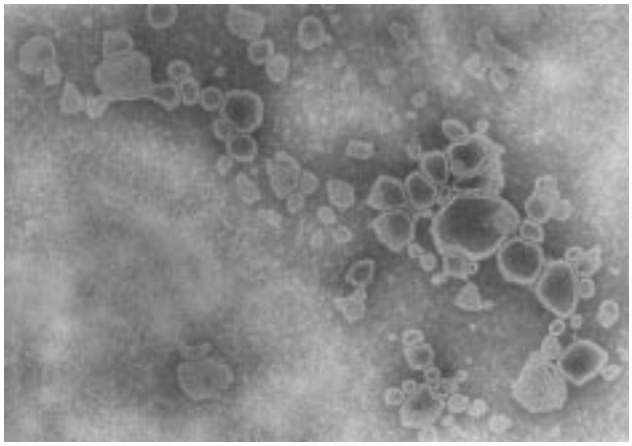


Fig. 7 Electron micrograph of UAC_8UA (1 cm = 55 nm)

pounds. A small amount of monomethyl ester may explain in this case the formation of vesicles.

Polydisperse multilayered vesicles (50–200 nm diameter) are also formed by UAC_8UA (Figs 7 and 8). The thickness of the layer is about 3.4 nm.

Conclusion

Three bolaamphiphiles with two urocanic acid heads groups have been synthesized. The shape and the size of aggregates in aqueous solution at various pH have been identified by light scattering and electron microscopy. Our results confirm the importance of the length of the alkyl chain and of pH on the aggregation behaviour of bolytes with carboxylic acid heads.

Experimental

Solvents were purchased from Prolabo or Carlo Erba and were used after drying and distillation. The reagents were purchased from Aldrich or Acros (>98% purity).

^1H and ^{13}C NMR spectra were recorded on Bruker AC 250 and AC 400 spectrometers. The DCI, NH_3 or CH_4 mass spectra were recorded on a Nermag R10-10 apparatus and the FAB mass spectra on a ZAB-MS apparatus (WG-Analytical, Manchester, UK). Infrared spectra were recorded on a Perkin Elmer 683b spectrophotometer and UV spectra on a Hewlett Packard 8452 A spectrophotometer. Melting points were determined on an Electrothermal apparatus (capillary tubes). The microanalyses were carried out at the ENSCT (Toulouse, France) on a Carlo Erba 1106 instrument.

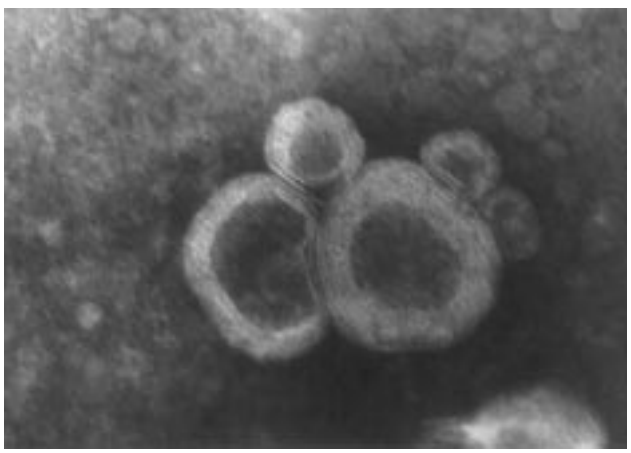


Fig. 8 Electron micrograph of UAC_8UA (1 cm = 35 nm)

Synthesis of urocanic compounds

(*E*)-Methyl urocanate **1** was synthesized according to the method of Lauth-de Viguerie and co-workers.[42]

Synthesis of UAC_{12}. A solution of 1-bromododecane (4.91 g, 19.70 mmol) in 10 ml of anhydrous THF was added dropwise to a mixture of **1** (1.50 g, 9.85 mmol), K_2CO_3 (13.6 g, 98.5 mmol) and 18-crown-6 (0.26 g, 0.985 mmol) in 25 ml of anhydrous THF. The mixture was stirred for 24 h at 60 °C. After filtration of the remaining K_2CO_3, the THF was removed by vacuum evaporation. The residue was recrystallised from hexane and then purified by flash chromatography (silica gel, chloroform–ethanol, 99 : 1, v : v). UAC_{12} methyl ester was obtained in 95% yield. Mp = 74.5 °C. $R_f = 0.65$; eluent CH_2Cl_2–EtOH (90 : 10, v : v). ^1H NMR ($CDCl_3$, δ): 0.87 (m, 3H, CH_3); 1.24 (s, 20H, CH_2); 1.70 (m, 2H, $CH_2\beta N$); 3.76 (s, 3H, CH_3O); 3.90 (t, $J = 7$ Hz, 2H, $CH_2\alpha N$); 6.52 (AB system, 1H, $J = 15$ Hz, CH=C*H*CO); 7.07 (s, 1H, H5im); 7.45 (s, 1H, H2im); 7.55 (AB system, 1H, $J = 15$ Hz, *CH*=CHCO). ^{13}C NMR ($CDCl_3$, δ): 168.14 (COO); 138.47 (C2im); 138.35 (C4im); 136.44 (*C*H=CHCO); 121.48 (C5im); 115.38 (CH=*C*HCO); 51.45 (CH_3O); 47.37 ($CH_2\alpha N$); 31.91 ($CH_2\beta N$); 30.95–22.69 (CH_2). Mass spectrum (DCI, NH_3): $m/z = 321$, MH^+ (100%). UV ($CHCl_3$): $\lambda_{max} = 290$ nm; $\varepsilon = 19413$ l mol^{-1} cm^{-1}. Anal. calcd (%) for $C_{19}H_{32}N_2O_2$, C 71.21; H 10.06; N 8.74. Found C 71.47; H 10.29; N 8.65.

A solution of UAC_{12} methyl ester (2.1 g, 6.55 mmol) and K_2CO_3 (9.05 g, 65.5 mmol) in a methanol–water mixture (40 ml : 20 ml) was heated at 40 °C for 24 h. After methanol evaporation, the solution was acidified (pH 5) and UAC_{12} obtained by precipitation in 75% yield. Mp = 175 °C. ^1H NMR [250 MHz, $(CD_3)_2SO$, δ]: 0.85 (t, $J = 6.5$ Hz, 3H, CH_3); 1.23 (m, 18H, CH_2); 1.77 (m, 2H, $CH_2\beta N$); 4.12 (t, $J = 7.1$ Hz, 2H, $CH_2\alpha N$); 6.67 (AB system, $J = 16$ Hz, CH=C*H*CO); 7.45 (AB system, $J = 16$ Hz, C*H*=CHCO); 8.04 (s, 1H, H5im); 9.02 (s, 1H, H2im). ^{13}C NMR [250 MHz, $(CD_3)_2SO$, δ]: 166.81 (COOH); 130.37 (C4im); 129.92 (*C*H=CHCO); 123.21 (C5im); 120.77 (CH=*C*HCO); 48.27 ($CH_2\alpha N$); 31.19 ($CH_2\beta N$); 29.28–21.99 (CH_2); 13.85 (CH_3). Mass spectrum (DCI, CH_4): $m/z = 307$, MH^+ (100%). IR υ(cm^{-1}): 3520 (OH); 3100 (=CH); 2930 (CH_2); 2860 (CH_3); 1680 (C=O); 1650 (C=C). UV (NaOH solution, pH = 13.2): $\lambda_{max} = 280$ nm, $\varepsilon = 13391$ l mol^{-1} cm^{-1}. Anal. calcd (%) for $C_{18}H_{30}O_2N_2 \cdot 2H_2O$, C 63.13; H 10.01; N 8.18. Found C 62.95; H 9.66; N 8.10.

Synthesis of the bolaamphiphiles UAC_8UA (5), $UAC_{12}UA$ (6) and $UAC_{16}UA$ (7). *1,16-dibromohexadecane.* To a stirred solution of *N*-bromosuccinimide (2.86 g, 16.1 mmol) in 50 ml of anhydrous THF at 0 °C, was added dropwise a solution of triphenylphosphine (4.22 g, 16.1 mmol) in 50 ml of anhydrous THF. After reaching room temperature a solution of hexadecane-1,16-diol (1.04 g, 4.02 mmol) in THF was added dropwise. The mixture was heated at 55 °C for an additional 2.5 h. The solvent was evaporated under vacuum. Water was added to the residue and the solution extracted with diethyl ether. The organic layer was washed with water, dried with $MgSO_4$ and evaporated under reduced pressure. Silica gel chromatography of the resulting solid (eluent: heptane) gave 1,16-dibromohexadecane (75% yield) as a white solid. Mp = 57.3 °C. $R_f = 0.60$; eluent: heptane. ^1H NMR ($CDCl_3$, δ): 1.26 (m, 24H, CH_2); 1.85 (quint, $J = 7$ Hz, 4H, $CH_2\beta Br$); 3.40 (t, $J = 7$ Hz, 4H, $CH_2\alpha Br$). ^{13}C NMR ($CDCl_3$, δ): 34.10 ($CH_2\alpha Br$); 32.86 ($CH_2\beta Br$); 29.63–28.20 (CH_2). Mass spectrum (DCI, CH_4): $m/z = 383$, MH^+ (25%); 303, $MH^+ - Br$ (100%). IR υ(cm^{-1}): 2841–2857 (CH_2). Anal. calcd (%) for $C_{16}H_{32}Br_2$, C 50.02; H 8.39. Found C 50.46; H 8.48.

Bolaamphiphile methyl esters. A solution of dibromoalkane (4.775 mmol) in 10 ml of anhydrous THF was added dropwise

to a mixture of **1** (1.45 g, 9.55 mmol), K_2CO_3 (13.3 g, 95.5 mmol) and 18-crown-6 (0.25 g, 0.955 mmol) in 25 ml of anhydrous THF. The mixture was stirred for five days at 60 °C. After filtration of the remaining K_2CO_3, the THF was vacuum-evaporated. The resulting product was purified by flash chromatography (eluent: dichloromethane–ethanol, 99 : 1 v : v) and then recrystallised from ethyl acetate–acetone.

UAC_8UA methyl ester. Yield = 52%. Mp = 138 °C. R_f = 0.67; eluent: CH_2Cl_2–EtOH (90 : 10). ^1H NMR ($CDCl_3$, δ): 1.20 (m, 8H, CH_2); 1.69 (quint, J = 7 Hz, 4H, $CH_2\beta N$); 3.69 (s, 6H, CH_3); 3.83 (t, J = 7 Hz, 4H, $CH_2\alpha N$); 6.46 (AB system, J = 15.6 Hz, 2H, CH=C*H*CO); 7.02 (s, 2H, H5im); 7.38 (s, 2H, H2im); 7.47 (AB system, J = 15.6 Hz, 2H, C*H*=CHCO). ^{13}C NMR ($CDCl_3$, δ): 168.23 (COO); 138.45 (C4im); 136.52 (*C*H=CHCO); 121.62 (C5im); 115.51 (CH=*CH*CO); 51.60 (CH_3O); 47.39 ($CH_2\alpha N$); 30.97 ($CH_2\beta N$); 28.97–26.48 (CH_2). Mass spectrum (DCI, NH_3): m/z = 415, MH^+ (100%). IR υ(cm^{-1}): 2877–2809 (CH_2); 1695 (C=O); 1635 (C=C). UV (EtOH): λ_{max} = 290 nm, ε = 44560 l mol^{-1} cm^{-1}. Anal. calcd (%) for $C_{22}H_{30}O_4N_4$, C 63.75; H 7.3; N 13.52. Found C 63.33; H 7.73; N 13.35.

$UAC_{12}UA$ methyl ester. Yield = 55%. Mp = 141 °C. R_f = 0.45; eluent: CH_2Cl_2–EtOH (90 : 10). ^1H NMR ($CDCl_3$, δ): 1.23 (m, 16H, CH_2); 1.74 (quint, J = 6.9 Hz, 4H, $CH_2\beta N$); 3.74 (s, 6H, CH_3); 3.88 (t, J = 6.9 Hz, 4H, $CH_2\alpha N$); 6.52 (AB system, J = 15.6 Hz, 2H, CH=C*H*CO); 7.06 (s, 2H, H5im); 7.45 (s, 2H, H2im); 7.52 (AB system, J = 15.6 Hz, 2H, C*H*=CHCO). ^{13}C NMR ($CDCl_3$, δ): 168.32 (COO); 138.9 (C4im); 136.45 (*C*H=CHCO); 121.62 (C5im); 115.65 (CH=*C*HCO); 51.67 (CH_3); 47.53 ($CH_2\alpha N$); 31.06 ($CH_2\beta N$); 29.50–26.61 (CH_2). Mass spectrum (FAB > 0, NBA matrix): m/z = 471, MH^+ (100%). IR υ(cm^{-1}): 3125 (CH=); 2941–2857 (CH_2, CH_3); 1700 (C=O); 1635 (C=C). UV (EtOH): λ_{max} = 290 nm, ε = 44448 l mol^{-1} cm^{-1}. Anal. calcd (%) for $C_{26}H_{38}O_4N_4, \cdot 2H_2O$, C 61.88; H 7.99; N 11.10. Found, C 62.14; H 7.73; N 10.97.

$UAC_{16}UA$ methyl ester. Yield = 65%. Mp = 153 °C. R_f = 0.35; eluent: CH_2Cl_2–EtOH (90 : 10). ^1H NMR ($CDCl_3$, δ): 1.22 (m, 24H, CH_2); 1.72 (quint, J = 7.0 Hz, 4H, $CH_2\beta N$); 3.73 (s, 6H, CH_3); 3.87 (t, J = 7.0 Hz, 4H, $CH_2\alpha N$); 6.51 (AB system, J = 15.7 Hz, 2H, CH=C*H*CO); 7.06 (s, 2H, H5im); 7.44 (s, 2H, H2im); 7.52 (AB system, J = 15.7 Hz, 2H, *CH*=CHCO). ^{13}C NMR ($CDCl_3$, δ): 168.32 (COO); 138.46 (C4im); 136.54 (*C*H=CHCO); 121.64 (C5im); 115.53 (CH=*C*HCO); 51.64 (CH_3O); 47.51 ($CH_2\alpha N$); 31.08 ($CH_2\beta N$); 29.75–26.63 (CH_2). Mass spectrum (DCI, NH_3): m/z = 527, MH^+ (100%). IR ν(cm^{-1}): 3100 (CH=); 2910–2840 (CH_2, CH_3); 1685 (C=O); 1630 ($C=C_{conj}$). UV (EtOH): λ_{max} = 290 nm, ε = 42879 l mol^{-1} cm^{-1}. Anal. calcd (%) for $C_{30}H_{46}N_4O_4 \cdot 1/2\ H_2O$, C 67.26; H 8.84; N 10.46. Found C 67.39; H 8.84; N 10.33.

Bolaamphiphiles UAC_8UA, $UAC_{12}UA$ and $UAC_{16}UA$. A solution of bolaamphiphile methyl ester (2.23 mmol) and K_2CO_3 (3.08 g, 22.3 mmol) in a methanol–water mixture (40 ml : 20 ml) was heated at 40 °C for 24 h. After vacuum evaporation of the methanol, the solution was acidified by 6 N HCl (pH = 5.7) and deprotected bolaforms precipitated.

Bolaamphiphile **5**, UAC_8UA. Yield = 80%. White solid, mp = 247 °C. ^1H NMR [400 MHz, $(CD_3)_2SO$, δ]: 1.21 (m, 8H, CH_2); 1.69 (quint, J = 7.1 Hz, 4H, $CH_2\beta N$); 3.94 (t, J = 7.1 Hz, 4H, $CH_2\alpha N$); 6.28 (AB system, J = 15.6 Hz, 2H, CH=C*H*CO); 7.41 (AB system, J = 15.6 Hz, 2H, C*H*=CHCO); 7.57 (s, 2H, H5im); 7.72 (s, 2H, H2im); 12 (s, 2H, COOH). ^{13}C NMR [400 MHz, $(CD_3)_2SO$, δ]: 167.87 (COOH); 136.85 (C4im); 136.60 (*C*H=CHCO); 122.67 (C5im); 114.88 (CH=*C*HCO); 46.17 ($CH_2\alpha N$); 30.13 ($CH_2\beta N$); 28.15–25.64 (CH_2). Mass spectrum (DCI, NH_3): m/z = 387, MH^+ (100%). IR υ(cm^{-1}): 3448 (OH); 3100–3070 (=CH); 2940 (CH_2); 2860 (CH_3); 1685 (C=O); 1640 (C=C). UV (NaOH solution, pH = 13.2): λ_{max} = 280 nm, ε = 42328 l mol^{-1} cm^{-1}. Anal. calcd (%) for $C_{20}H_{27}O_4N_4Cl \cdot 1/2H_2O$, C 55.6; H 6.53; N 12.97. Found C 55.90; H 6.94; N 12.97.

Bolaamphiphile **6**, $UAC_{12}UA$. Yield = 70%. White solid, mp = 227 °C. ^1H NMR [400 MHz, $(CD_3)_2SO$, δ]: 1.21 (m, 16H, CH_2); 1.70 (quint, J = 7.0 Hz, 4H, $CH_2\beta N$); 3.94 (t, J = 7.0 Hz, 4H, $CH_2\alpha N$); 6.27 (AB system, J = 15.5 Hz, 2H, CH=C*H*CO); 7.40 (AB, J = 15.5 Hz, 2H, C*H*=CHCO); 7.57 (s, 2H, H5im); 7.70 (s, 2H, H2im). ^{13}C NMR [400 MHz, $(CD_3)_2SO$, δ]: 167.92 (COOH); 136.98 (C4im); 136.63 (*C*H=CHCO); 122.62 (C5im); 114.88 (CH=*C*HCO); 46.15 ($CH_2\alpha N$); 30.18 ($CH_2\beta N$); 28.72–25.72 (CH_2). Mass spectrum (DCI, NH_3): m/z = 443, MH^+ (100%). IR υ(cm^{-1}): 3571 (OH); 3125 (CH=); 2940 (CH_2); 2860 (CH_3); 1681 (C=O); 1653 (C=C). UV (NaOH solution, pH = 13.2): λ_{max} = 280 nm; ε = 40533 l mol^{-1} cm^{-1}. Anal. calcd (%) for $C_{24}H_{36}O_5N_4$, C 62.59; H 7.88; N 12.16. Found C 62.22; H 7.72; N 11.80.

Bolaamphiphile **7**, $UAC_{16}UA$. Yield = 70%. White solid, mp = 201 °C. ^1H NMR [400 MHz, $(CD_3)_2SO$, δ]: 1.20 (m, 24, CH_2); 1.72 (quint, J = 7.0 Hz, 4H, $CH_2\beta N$); 4.02 (t, J = 7.0 Hz, 4H, $CH_2\alpha N$); 6.47 (AB system, J = 15.8 Hz, 2H, CH=C*H*CO); 7.42 (AB, J = 15.8 Hz, 2H, C*H*=CHCO); 7.78 (s, 2H, H5im); 8.30 (s, 2H, H2im). ^{13}C NMR [400 MHz, $(CD_3)_2SO$, δ]: 167.39 (COOH); 134.01 (C4im); 133.63 (*C*H=CHCO); 122.86 (C5im); 117.49 (CH=*C*HCO); 47.09 ($CH_2\alpha N$); 29.80 ($CH_2\beta N$); 28.91–25.60 (CH_2). Mass spectrum (DCI, NH_3): m/z = 499, MH^+ (100%). IR υ(cm^{-1}): 3400 (OH); 3100 (=CH); 2910 (CH_2); 2840 (CH_3); 1690 (C=O); 1635 (C=C). UV (NaOH solution, pH = 13.2): λ_{max} = 280 nm, ε = 38530 l mol^{-1} cm^{-1}. Anal. calcd (%) for $C_{28}H_{48}O_6N_4Cl_2$, C 55.35; H 7.96; N 9.22. Found C 55.88; H 7.68; N 9.14.

Molecular aggregation of the bolaforms in aqueous solution

Surface tension measurements were made with a Prolabo Tensiomat 3 (equipped with a thermostated water bath; T = 30 °C) using the stirrup detachment method. Conductivity measurements were made at 30 °C with a conductivity–resistivity meter CDRV62 (Tacussel Electronique) with a platinium electrode. UV absorbance measurements were made with a Hewlett Packard 8452A spectrometer. A Coulter N4MD was used for the light scattering measurements. The electron micrographs were obtained using a EM-301 PHILIPS electron microscope. The samples were prepared by negative staining with uranyl acetate on a carbon film.

Vesicles were prepared by the sonication method with a titanium probe (High Intensity Ultrasonic Processor 600-Watt Model). Solutions of 10^{-3} mol l^{-1} were sonicated at 0 °C for 15 min using an 80% duty cycle. Titanium (from the probe) and dust were removed by centrifugation (3000 rpm^{-1} for 10 min) and filtration through a Millipore 0.45 μ filter.

References

1 H. A. Morrison, *Photodermatology*, 1985, **2**, 158.
2 N. K. Gibbs, M. Norval, N. Traynor, M. Wolf, B. E. Johnson and J. Crosby, *Photochem. Photobiol.*, 1994, **60**, 280.
3 W. Schwartz, K. Langer, H. Schell and A. Schonberger, *Photodermatology*, 1986, **3**, 239.
4 A. Zenisek and J. A. Kral, *Biochem. Biophys. Acta*, 1953, **12**, 479.
5 P. M. Krein and D. Moyal, *Photochem. Photobiol.*, 1994, **60**, 280.
6 J. H. Anglin Jr. and W. H. Batten, *Photochem. Photobiol.*, 1970, **11**, 271.
7 H. A. Morrison and R. M. Deibel, *Photochem. Photobiol.*, 1988, **48**, 153.
8 S. J. Farrow, C. R. Jones, D. L. Severance, R. M. Deibel, W. M. A. Baird and H. A. Morrison, *J. Org. Chem.*, 1990, **55**, 275.
9 E. C. De Fabo and F. P. Nooman, *J. Exp. Med.*, 1983, **158**, 84.
10 M. Norval, N. K. Gibbs and J. Gilmour, *Photochem. Photobiol.*, 1995, **62**, 209.
11 M. Norval, T. J. Simpson and J. A. Ross, *Photochem. Photobiol.*, 1989, **50**, 267.

12 K. Kinito, K. Punnonen, J. Toppain and L. Leino, *Inflammation*, 1996, **20**, 451.
13 A. M. Moodycliffe, C. D. Bucana, M. L. Kripke, M. Norval and S. E. Ullrich, *J. Immunology*, 1996, **157**, 2891.
14 M. C. Monje, A. Lattes and M. Rivière, *Bull. Soc. Chim. Fr*, 1990, **127**, 292.
15 J. H. Fuhrhop and J. Mathieu, *Angew. Chem., Int. Ed. Engl.*, 1984, **96**, 124.
16 R. Nagarajan, *Chem. Eng. Commun.*, 1987, **55**, 251.
17 F. M. Menger and S. Wrenn, *J. Phys. Chem.*, 1974, **78**, 1387.
18 S. Yiv, K. M. Kale, J. Lang and R. Zana, *J. Phys. Chem.*, 1976, **80**, 2651.
19 S. Yiv and R. Zana, *J. Colloid Interface Sci.*, 1980, **77**, 449.
20 R. Zana, S. Yiv and K. M. Kale, *J. Colloid Interface Sci.*, 1980, **77**, 456.
21 Y. Okahata and T. Kunitake, *J. Am. Chem. Soc.*, 1979, **101**, 5231.
22 J. H. Fuhrhop and D. Fritsch, *Acc. Chem. Res.*, 1986, **19**, 130.
23 Z. Mirghani, D. Bertoia, E. Piano and A. Gliozzi, *Biochem. Soc. Trans.*, 1989, **17**, 507.
24 J. H. Fuhrhop, H. H. David, J. Mathieu, U. Liman, H. J. Winter and E. Boekema, *J. Am. Chem. Soc.*, 1986, **108**, 1785.
25 J. H. Fuhrhop and R. Bach, *Advances in Supramolecular Chemistry*, 1992, **2**, 25.
26 R. Garelli-Calvet, F. Brisset, I. Rico and A. Lattes, *Synth. Commun.*, 1993, **23**, 35.
27 F. Brisset, R. Garelli-Calvet, J. Azéma, C. Chebli, I. Rico-Lattes and A. Lattes, *New J. Chem.*, 1996, **20**, 595.
28 C. Madelaine-Dupuich, B. Guidetti, C. Rico-Lattes and A. Lattes, *New J. Chem.*, 1996, **20**, 143.
29 P. Böhme, H. G. Hicke, C. Boettcher and J. H. Fuhrhop, *J. Am. Chem. Soc.*, 1995, **117**, 5824.
30 J. A. McDonald, S. A. Butler and A. R. Rennie, *Colloids Surf. A*, 1995, **102**, 137.
31 M. Jorgensen and K. Bechgaard, *J. Org. Chem.*, 1994, **59**, 5877.
32 G. R. Newkome, C. N. Moorefield, G. R. Baker, R. K. Behera G. H. Escamillia and M. J. Saunders, *Angew. Chem., Int. Ed. Engl.*, 1992, **31**, 917.
33 M. Ishikawa, K. I. Matsumura, K. Esumi, K. Meguro, W. Binana-Limbele and R. Zana, *J. Colloid Interface Sci.*, 1991, **151**, 70.
34 N. Jayasuriya, S. Bosak and S. L. Regen, *J. Am. Chem. Soc.*, 1990, **112**, 5844.
35 D. Tsiourvas, C. M. Paleos and A. Malliaris, *Progr. Colloid Polym. Sci.*, 1994, **97**, 163.
36 E. K. Comeau, E. J. Beck, J. F. Caplan, C. V. Howley and D. G. Marangoni, *Can. J. Chem.*, 1995, **73**, 1741.
37 R. Festag, V. Hessel, P. Lehmann, H. Ringsdorf and J. H. Wendorff, *Recl. Trav. Chim. Pays Bas*, 1994, **113**, 222.
38 G. Mao, Y. Tsao, M. Tirrell and H. T. Davis, *Langmuir*, 1993, **9**, 3461.
39 A. Kokkinia, C. M. Paleos, A. Malliaris and A. Xenakis, *Progr. Colloid Polym. Sci*, 1993, **93**, 302.
40 R. Sköld and M. A. R. Tunius, *J. Colloid Interface Sci.*, 1992, **152**, 183.
41 J. H. Fuhrhop, D. Spiroski and C. Boettcher, *J. Am. Chem. Soc.*, 1993, **115**, 1600.
42 N. Lauth-de Viguerie, N. Sergueeva, M. Damiot, H. Mawlawi, M. Rivière and A. Lattes, *Heterocycles*, 1994, **37**, 1561.
43 K. Meguro, K. Ikeda, A. Otsuji, M. Taya, M. Yasuda and K. Esumi, *J. Colloid Interface Sci.*, 1987, **118**, 372.
44 J. A. McDonald J. A., Butler S. A. and A. R. Rennie, *Colloids Surf. A: Physicochem. Eng. Aspects*, 1995, **102**, 137.
45 F. Menger and K. D. Gabrielson, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 2091.
46 H. A. Hofland, J. A. Bouwtra, G. S. Gooris, F. Spies, H. Talsma and H. E. Junginger, *J. Colloid Interface Sci.*, 1993, **161**, 366.
47 J. H. Fuhrhop, U. Liman and V. Koesling, *J. Am. Chem. Soc.*, 1988, **110**, 6840.
48 D. D. Lasic, *Biochem. J.*,1988, **256**, 1.
49 H. H. Hub, U. Zimmerman and H. Ringsdorf, *FEBS Lett.*, 1982, **140**, 254.
50 R. W. de Groot, A. Wagenaar, A. Sein and J. B. F. N. Engberts, *Recl. Trav. Chim. Pays Bas*, 1995, **114**, 371.
51 Jaeger D. A. and Brown E. L. G., *Langmuir*, 1996, **12**, 1976.
52 D. P. Cistola, J. A. Hamilton, D. Jackson and D. M. Small, *Biochemistry*, 1988, **27**, 1881.
53 K. Edwards, M. Silvander and G. Karlsson, *Langmuir*, 1995, **11**, 2429.

Received 28*th May* 1997; *Paper* 7/08326B